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EDITORIAL

PHENOTHIAZINE AS AN ANTHELMINTIC

THE attention of readers is drawn to an abstract and the conclusions of a report* published in this number of the VETERINARY JOURNAL. The report is a description of a carefully planned co-ordinated piece of work the results of which were examined by statistical methods and from which an authentic statement can be made of the value of phenothiazine as an anthelmintic for sheep. Phenothiazine since its introduction into this country has been used in increasing amounts in the treatment of parasitic infestations of the alimentary tract of domestic animals, especially sheep, and a considerable number of reports in the literature give strong evidence of its efficacy. It was necessary, however, to carry out trials of the type described in the above-mentioned report in order to obtain convincing evidence on the results of its use. The Agricultural Research Council arranged the trials and four groups of workers at four centres participated in them. The trials were so arranged that similar techniques and methods were adopted by each group of workers with the result that suitable comparisons could be made and the four trials treated as a whole. The work is an example of the value of careful planning and statistical analysis in assessing the results of an experiment. Too often do we read of experiments and trials carried out with biological products and drugs in the prevention and treatment of diseases of live stock, the results of which seem to indicate that they are of

*Co-ordinated trials with phenothiazine against nematodes in lambs. Imperial Agricultural Bureaux Joint Publication No. 4. April, 1943.

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value without leaving the reader with a firm conviction. The carefully planned experiment with statistically analysed results leaves no doubt on the subject.

We know now from this report that phenothiazine is a satisfactory anthelmintic as far as sheep are concerned, the types of helminths on which it is effective, and the dose range at which it should be administered to produce satisfactory results. It is of interest to note, for example, that a dose as small as 5 gm. given to sheep kept under conditions to prevent reinfestation, had a very marked effect on *Hæmonchus*, and that in sheep kept outside, the increase in weight of the dosed animals increased roughly proportionately to the amounts of phenothiazine used. This would seem to indicate that the larger the dose the more was the effect on the worms in the small intestine—worms which hitherto may not have been considered of great clinical importance. It is also shown from the analysis of the results that the somewhat commonly held belief that heavily infested lambs respond less well to the drug than do lightly infested animals may be a misconception.

The report deals only with sheep. There is evidence from other sources that phenothiazine exerts beneficial results in the treatment of parasitic infestations of other animals, especially horses and pigs. The results in horses indicate that it is of much value in the treatment and prevention of strongylosis. Attention has been drawn, however, to the few unhappy results which have followed its administration even in moderate doses. Taylor (*Vet. Rec.*, 1942, liv, 95) gave a warning of the sensitivity of some horses to the effects of phenothiazine and recommends the exercise of caution in treating horses, especially anæmic animals. He suggests that if a group of horses is to be treated it is wise to use the drug on a small number before the whole group is dosed in order to ascertain whether any sensitivity exists in the group. Again, in the treatment of pigs, Lapage (*Vet. Rec.*, 1943, lv, 184), for example, discusses symptoms of illness which may follow the dosing of young pigs with phenothiazine. In the case of pigs it may be that individual dosing would prevent the occurrence of these symptoms to some extent; for there is little doubt that by mass dosing in the feed some pigs receive a greater quantity of the drug than do others.

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General Articles

TESTICULAR GRAFTING IN DOMESTIC ANIMALS

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For very many centuries the testicles have been known as the source of individual male sexual virility, and to procure the loss of such virility, together with other effects, man has long practised castration upon his own kind and upon animals.

Removal of the testicles produces some well defined changes in the subjects, particularly if the removal is made before puberty, in which case the body's subsequent development expresses conspicuous female temperamental and structural characteristics. It is now also known that total loss of testicular function is followed by hypertrophy of the pituitary gland's anterior lobe, pineal and thyroid atrophy and derangement of suprarenal activity, causing genital dystrophia.

In the second half of the 19th century, the essential influences of the testes on the whole organism began to be better understood, and during the past thirty years, by the introduction of testicular implantations and transplantations, considerably more on the subject has been revealed. The first implantation of cockerel's testicles into a hen were carried out by Hunter in 1767, and his experiment was repeated later by many physiologists. Pézard also implanted testicles into hens and castrates and observed the hens developed a cock's comb and the castrates recovered all their male characters. It is to be noted that implantations into poultry "take" well, because avian tissues transmit the character of embryonal cells and the implantations grow more rapidly and easily than mammalian tissues under similar conditions.

In 1909, the famous Viennese physiologist, Steinach, performed many testicular implantations into rats under the name "feminatio and masculinatio," and succeeded in producing hermaphrodites. The implantation amounted to whole or part of the testis being imbedded subcutaneously, intramuscularly, or attached to the parietal peritoneum. Steinach, moreover, having investigated the development of testicular endocrinal cells, performed the rejuvenation of an emaciated and hairless senile rat after section or ligation of the vas deferens.

Steinach's experiments repeated by Zawadowski, Lipschütz, Nusbaum-Hilarowicz, Payr, *et alia*, were decisive and provided foundation for recognition of the essential cause of puberty and testicular endocrinal activity. Further, these experiments provided the first evidence that the function of the testicles was not only to create spermatozoa, but also to determine the secondary sexual characteristics of the male.

Still, long before the discovery of sexual hormones, the ingenious Frenchman, Séquard maintained that testicular secretion contained the substances stimulating the functions of most organs and tissues, especially brain muscle and various glands; when an old man injected himself subcutaneously with an extract from animal testes he described their invigorating action.

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The testicles of mammals if implanted in the peritoneal cavity, subcutaneously or intramuscularly, survive only a short time, and after several weeks undergo degeneration and resorption. The easy execution of Steinach's ligation or resection of vas deferens resulted in its application to man, but the effects were transient.

The investigations and implantations so far described, although very important scientifically, were largely confined to the territory of physiological experimentation, rarely practicable, and with ephemeral results.

Then came a completely different surgical method of rejuvenation by Serge Voronoff, Professor of the College of France. This method was first communicated by Voronoff to the Congrès Français de Chirugie in 1923. The communication, illustrated with photographs, told in accurate detail of the results following grafting operations in men, rams and he-goats. Later, Voronoff's co-workers—Baudet, Dartigues, Didry, and others—published similar accounts.

Voronoff, on the basis of his testicular grafting, maintained that although the primary function of the testicles is to assure the expression of genital virility, it is, however, a fact that the role of the testis as the stimulator of vital energy, may prove to be even more important than its role as the stimulator of sexual energy.

Voronoff's interpretation is as follows: "This testicular secretion varies both in quantity and quality in different individuals, as it does at different periods of life. Those subjects who are vigorous and energetic with a lively imagination, a capacity for work and the power of resisting fatigue, possess highly active testicles which contribute their vivifying principle in abundance to the blood stream. Should this source of energy be diminished by age or disease the body becomes enfeebled, the blood impoverished, the brain starved and, in fact, all the physical forces become impaired."

Voronoff knew that the life of every organ depends upon a nutrient blood supply but, that the blood supply of the testes is largely through vessels so small, that their anastomosis with vessels of a testicular graft is impossible. Aware of Carrel's discovery that tissues can be kept alive *in vitro* for years in blood serum, and of the well known phenomenon of exudation of blood plasma, after the provocation by traumatism or irritation of tissues, Voronoff chose the introduction with local scarification, of thin slices of the donor's testicle into the tunica vaginalis of the recipient's testicle. He believed that this precaution of scarification could not fail to provoke an extravasation of serum sufficient to maintain life in the graft until the new vascularisation was established. The method would have the further advantage of placing the graft in the very situation which it was destined by nature to occupy. The correctness of Voronoff's supposition was demonstrated not only by the satisfactory clinical results after testicular grafting in animals and later in men, but also by the precise histological examinations of the grafts by Retterer, Professor and Fellow of the Faculté de Médécine in Paris, who communicated the results of these histological examinations to the Société de Biologie of Paris in 1919-22. Each month Retterer examined histologically the grafts from operated rams and stated irrefutably that "After a short period the grafts showed a certain amount of degeneration of the epithelial lining of the semini-

ferous tubules, the majority of which had collapsed. The internal coat of the tubules was not only living after some months to a year, but had increased to such an extent that it filled the lumen of the tubules converting them into cords."

The living and active slices of testicles transplanted by Voronoff's method bestowed upon the host qualities and characteristics determined by the nature of secretion, which its cells continued to elaborate, supplied new stimulants, assured renewed activity to the various organs and continuously provided the host with a reserve of sexual hormone for a long time, or, at least, for as long as the cells would have continued their activities in the body of their original host.

The first experiments were made by Voronoff on rams of 12 to 14 years old, manifesting all the symptoms of late senility. Into the tunica vaginalis of these rams, Voronoff grafted slices of testicles obtained from 2- to 3-year-old rams. After several weeks the old rams became progressively more vital and energetic, grew a thick, new wool and the animals' general physical condition improved. At a time some 12 to 18 months after the symptoms of rejuvenation were at their height, Voronoff removed the engrafted slices of testicle and found that 3 to 5 months later the rams redeveloped all the symptoms of senility. This was sufficient evidence of the removed grafts' previous activity and the efficacy of this method of testicular grafting.

Voronoff next applied this method to the rejuvenation of men senile with years or prematurely old. The outstanding problem, however, was to obtain suitable grafts. In rams, transplantations of testis between individuals of the same species (autograft) had yielded good results and the supply of material from other young rams presented no difficulty, but the supply of a young human testis (homograft) was a very different matter and even opportunities offered by fatal accidents were precluded by law.

Transplantation of ram's or of most other animals' testicles to man (heterograft) is unprofitable and dangerous since the grafts act as toxic foreign bodies and fail to coalesce. Voronoff, therefore, used slices of testicle from anthropoid apes (chimpanzee, orang-utan, and gorilla) these animals having serological and other biological relationship with homo sapiens.

Over one hundred such grafting operations on men, among whom were seven physicians, were carried out with testicular tissue from young adult apes. The men, as in the case of rams similarly treated, derived a general increase in their physical and mental energy as well as a resumption of sexual activity. Physical and mental rehabilitation was, according to Voronoff, absolute in 36 to 88 per cent. of cases, while in 26 to 55 per cent. it was accompanied by complete restoration of sexual potency.

Voronoff's method of testicular transplantation can have a large economic importance in the husbandry of domestic animals, and by the operation it is possible to restore virility to the old and more valuable breeding male and so increase its utility. The French Government in Algeria and Tunisia arranged several farms where Voronoff's experiments were performed on great numbers of rams with very satisfactory results. Subsequently, in Poland, the method with my modification was employed in many aged stallions, bulls and dogs. The Voronoff technique was to cut the testicle of the donor into six slices,

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each then being grafted at appropriate distances apart into the external surfaces of the parietal folds of both tunicæ vaginalis.

A better result entailing more rapid and abundant graft growth was obtained by me in 1926-1939 after transplantation of testicle slices from young donors, into a pocket under the tunica albuginea after its separation from parenchyma, to old recipients. The improved result applied especially to the rejuvenation of old stallions—the effects of which were well marked. In most cases, before the operation the stallions were in a state of emaciation, trembling in their extremities and capable of rising only with difficulty.

Their appearance was sluggish, the coat poor and they were markedly apathetic to their environment and in the presence of mares. Four weeks after grafting these previously senile animals began to revitalise and became energetic and even aggressive. The first symptom of graft's action was a very abundant and sudden shedding of the old hair. This was followed by the growth of a shining coat together with a general reanimation. Contrary to results obtained in man, all the stallions developed a strong libido sexualis and were able to cover mares successfully.

Almost entirely similar were the symptoms of rejuvenation in old dogs. Those in bulls, however, were less significant and, appearing later than in stallions, were marked chiefly by the return of the sexual impulse. In the equine cases testicular tissue was obtained from donors of 1½ to 3 years old, in the bovine, and in canine cases, 1 to 2 years in the course of routine castrations. Best results were given when donor and recipient were not only of the same breeding but of near relationship.

The operation on stallions is performed in the following manner:—

Slices of testicular parenchyma (about ½ cm. thick, 2 cm. wide and 3 cm. long), after removing tunica albuginea, are cut from the castrated testicles. The epididymis is removed. To rejuvenate one old stallion it is sufficient to graft one slice of parenchyma from a young stallion (donor) into one of the testicles of the old stallion (recipient). One may also graft the transplantates into both testicles of the old stallion, this, however, would seem to be unnecessary as it should be better to reserve the other non-grafted testicle of the old stallion for eventually grafting it in the event that the first operation should prove to be unsuccessful, or to carry out the operation for the second time, when the good results of the first transplantation begin to disappear.

Hardening of both or one of the testicles occurs sometimes with old stallions. Stallions with both testicles sclerotic cannot be rejuvenated. If only one testicle is hardened and the second is normal, the operation should take place on the healthy testicle.

The grafting of the transplantate from donors should take place as soon as possible after castration, so long as the cells of the transplantate are alive. Therefore, the castration of the donor is carried out at the same time as the operation on the recipient. One of the surgeons performs the castration and the second operates on the old stallion. The old stallion is cast and narcotised. Then the young stallion is cast and castrated. Slices of the testicles are cut and placed in a container with 0.9 per cent. of physiological solution NaCl, at 38 deg. to 40 deg. C. temp.

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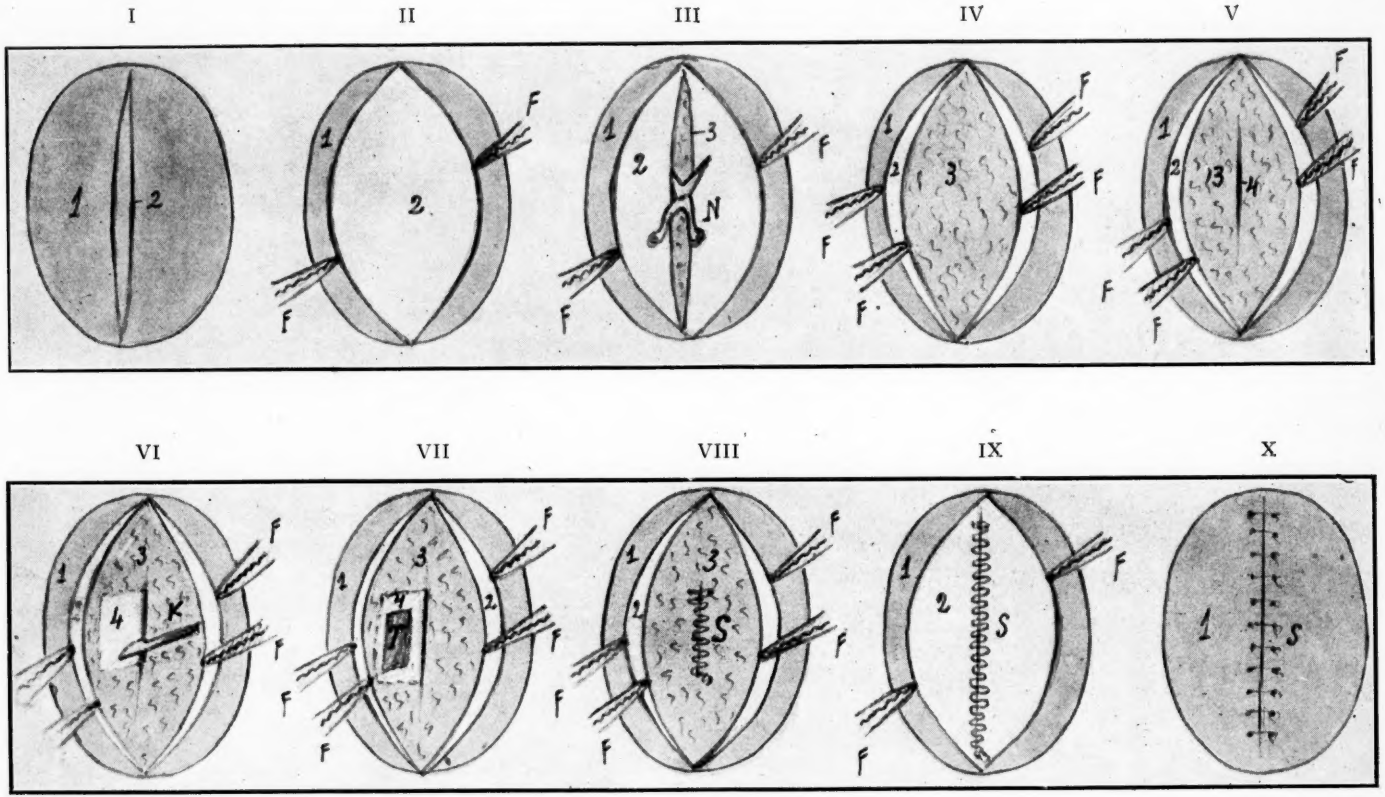
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Immediately after the transplantates are prepared the surgeon grafts them into the testicles of the old stallion. This usually does not take more than five to ten minutes.

The old stallion is put down by an intravenous injection of chloral hydrate and with the hind legs extended backwards and tied to posts.

The forelegs are tied up in the usual way. Both sides of the stallion are supported by stuffed sacks. The scrotum and adjacent areas are disinfected with 50 to 60 per cent. of alcohol or methylated spirit and covered with sterilised linen.

The following instruments are necessary for the operation: a convex knife, straight scissors, several forceps, needles, catgut and silk.

The operation may be described in ten phases (see diagram):—

(I) The left or right testicle is taken hold of with the fingers of the left hand and the skin of the scrotum stretched. A careful incision is made alongside the scrotum about 6-7 cm. long, depending on the size of the testicle.

(II) The edges of the incised scrotum are fixed with two forceps to expose the tunica vaginalis communis.

(III) Tunica vaginalis communis is cut carefully with scissors. This incision should be a little smaller than that of the scrotum (5 to 6 cm.), depending on the size of the testicle. The skin of the scrotum and the tunica vaginalis communis may also be cut at the same time (as usually during castration); this, however, is not advisable, as then the testicle itself may be cut deeply, causing hæmorrhage and thus obscuring and soiling the operational field.

(IV) The edges of the incised tunica vaginalis communis are now fixed with forceps in order to expose to sufficiently wide area of the tunica albuginea about the centre of the testicle.

(V) Tunica albuginea is incised carefully (for about 3½ to 4 cm.).

(VI) Tunica albuginea is separated carefully with the sharp end of the knife from the parenchyma of the testicle, in order to make a sufficiently wide pocket for the transplantate. When preparing the tunica albuginea one should see that as little as possible of the parenchyma is damaged in order to avoid unnecessary bleeding.

(VII) The prepared transplantate (size ½ x 2 x 3 cm.) is taken out with forceps from the physiological solution of NaCl and placed into the pocket of tunica albuginea.

(VIII) After placing the transplantate, the edges of the pocket of tunica albuginea are sewn with catgut (continuous suture).

(IX) The testicle is covered with tunica vaginalis communis by lifting the forceps fixed to the edges of the tunica vaginalis communis. Tunica vaginalis communis is also sewn with catgut in a continuous suture.

(X) The forceps fixed to the edges of the incised scrotum are lifted, the testicle is covered with the scrotum and the edges of the incision are closed by interrupted silk sutures.

Strict aseptic precautions must be taken throughout the operation. The skin wound should not be disinfected, but may be covered with a roll of cotton-wool sewn to the skin of the scrotum.

The operation on old dogs is carried out in a similar manner. The donors

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are young dogs (1 to 2 years old), of the same breed, or, better still, related to the recipient. The dog is anæsthetised with nembutal, intravenously. With old bulls in which casting may be contraindicated, grafting may be performed with ease on a standing bull by tying his hind legs and tail after a local novocain anæsthetic of the testicle.

The operator stands behind the bull. The testicles are held in the same manner as during castration of the standing bull, but the assistant should hold the testicle, very tightly.

The transplantates are taken from young bulls (1 to 2 years old). Beyond a more or less œdema of the testicles, no other complications after an aseptic operation should occur. The wound heals *per primam* and the sutures of the scrotum should be removed in about seven to nine days after the grafting.

The effect of operation is maintained for from one to two years, at the end of which time transplantation may be repeated, the second graft being as efficacious as the first.

Testicular grafting in domestic animals remains a surgical means of rejuvenation in which mortality is nil. The effects by comparison are most enduring and the results positive in a high percentage of cases; for economic purposes, especially after this war, it deserves a greater popularity.

Testicular grafting is not and will not be a panaceum for all the difficulties of animal breeding associated with the senile male impotence. It should be remembered that Voronoff himself said: "Grafting cannot recall the dead."

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SPIROCHÆTOSIS OF FOWLS IN INDIA—II

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Methods of Immunisation, Treatment and Immunity.

The prevention of spirochætosis consists in taking measures to eliminate ticks, a policy which can be adopted on organised poultry farms owned by educated and well-to-do keepers. In India such farms are few and almost the whole poultry industry, if it can be so termed, is in the hands of villagers. Attempts to give advice on poultry-keeping and hygiene to such persons are usually fruitless. Obviously, therefore, in India it is more suitable to adopt such measures as immunisation and treatment of birds. Investigators in different parts of the world have attacked these problems in different ways and a number of such methods have been reported, though some are supported by few experimental data. The only agreed fact is that after an attack birds are strongly immune. As regards the duration of such immunity, again there is difference of opinion. The only work on methods of immunisation and their possible application in the field and on duration of immunity so far carried out on an adequate scale is that of Kliger, Hermoni and Perek (1938) in Palestine.

Gabritschewsky (1938) was the first to make serological studies on the disease. He prepared a prophylactic serum from a horse by immunising it with massive doses of goose serum containing spirochætes. He claims that in geese the period of passive immunity, following a dose of not less than 2 c.c. of this serum, ranged from twenty to thirty days.

Marchoux and Salimbeni (1903) prepared a vaccine with fresh fowl serum and virulent blood. After forty-eight hours' exposure to the serum the spirochætes were dead and the serum-blood, when injected into a fowl forty-eight hours before artificial infection, prevented the disease. They also found that serum from a recovered fowl prevents infection when given twenty-four hours before an injection of virulent blood.

Levaditi (1904) found that 2 to 4 c.c. inactivated convalescent serum, injected into a fowl on the third, fourth or fifth day of the disease, causes severe symptoms and death, due to capillary embolism following agglutination of spirochætes, and agglomeration of leucocytes. On a healthy bird, the serum had no such effect. The occurrence of this phenomenon has been corroborated by Neufeld and Prowazek (1907) who further report that the disease is prevented by injecting 0.0025 c.c. of immune serum into a fowl twenty-four to forty-eight hours before infection. Virulent blood containing spirochætes killed by the addition of sodium taurocholate is said to be useful as a vaccine.

Dschunkowsky and Luhs (1909) obtained a curative and preventive vaccine from the serum of recovered geese and claim that injection of 0.01 gm. of this serum protected against the disease and 5 gm. proved curative.

Aragao (1911) prepared a vaccine by formalinising infected fowl's blood. The vaccine is said to retain its efficacy for thirteen months and 1 c.c. of it given subcutaneously confers immunity. He also prepared a serum from goats, which was said to protect a bird against the disease when injected half an hour before infection in dose of 0.5 c.c., in dose of 1 c.c. simultaneously or when 10 c.c. was given twenty-four hours after infection.

Aoki (1914) found that the antigenic properties of the spirochætes were not injured by (*a*) drying at 50 deg. cent. for twenty-four hours, (*b*) through washing with saline, followed by refrigeration for a month, (*c*) treatment with chloroform.

Hoffmann (1921) found that the blood of birds recovered from the disease is protective against infection for three to four weeks and that there is hereditary transmission of immunity in fowls.

Verge (1923) successfully immunised fowls with infected blood which had been kept for sixty days or heated at 55 deg. cent. for from five to ten minutes.

Morcos (1935) prepared a vaccine by emulsifying the liver and spleen of infected birds in glycerine saline, preserved in formalin and kept for ten days.

Knowles *et al.* (1932) recommended 2 c.c. of immune serum injected twenty-four hours before infection as a prophylactic against the disease. Their results are, however, based on work with only a small number of birds. They report that the birds acquire a strong immunity but fail to mention the duration thereof.

Although some success has attended the use of serum and vaccines, such methods are less frequently employed than chemotherapy, e.g., with atoxyl and soamin.

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Uhlenhuth, Gross and Bickel (1907) used atoxyl in 5 cgm. doses on fowls against spirochætosis and found it efficacious. Levaditi and McIntosh (1907) made a close study of the therapeutic effect of atoxyl and found the action to be indirect by stimulation of phagocytosis and the bringing about of a "precocious crisis." For years, atoxyl had been the agent chiefly used as a spirochæticide. Recently, other arsenical preparations have been introduced for the purpose but no tests of the comparative value of such compounds have been carried out.

Dschunkowsky and Luhs (1909) used 0.3 to 0.5 gm. of atoxyl two hours before infection and obtained prophylactic effects. Doses of 0.3 to 0.4 gm., when injected at twenty-four-hour intervals from the third to fifth day of the disease, proved curative.

Dodd (1910), in Queensland, found soamin to be very efficient in the treatment of spirochætosis.

Leese (1912) treated successfully spirochætosis in Jhelum and Sohawa with soamin. One-eighth gr. was given to adult Indian fowls and 1/10 gr. for pullets and chicks. In many cases, however, a second dose was required.

Macfie and Johnston (1914), in Southern Nigeria, treated three natural cases of spirochætosis in fowls by injecting 3/50 gr. atoxyl. Two of the birds recovered with the first injection but the third died in spite of repeated injections. Two others were treated with 0.002 gm. of salvarsan and, although both recovered, one continued to show spirochætes for three days. Yakimoff, Schokhor and Koselkine (1916), in Russian Turkestan, found atoxyl in doses of 0.06 to 1 gm. to adult fowls and 0.03 to 0.05 gm. to chickens gave excellent results.

Wirth and Langer (1937) experienced mortality of 50 per cent. after treatment with atoxyl, 0.05 gm. per kilogram live weight. All the dead birds showed visceral gout. Atoxyl is contraindicated in such cases, owing to the severe intoxication which follows.

At the beginning of this investigation the most popular method of immunisation was followed, i.e., injecting 0.25 c.c. of virulent blood and twenty-four hours later injecting 0.03 gm. of atoxyl per kilogram body-weight intramuscularly. This was not found to be very satisfactory owing, presumably, to the drug having been given too early.

The present experiments were conducted in order to establish a safe method of immunising fowls against spirochætosis and also to collect information on the spirochæticidal action and dosage of various arsenical preparations available in the market. During this enquiry more than 2,000 fowls, mostly Rhode Island Reds and White Leghorns, were used. Most of these were over six months of age.

The work may be considered under the following headings:—

(1) Methods of immunisation.
   (*a*) Refrigerated blood as vaccine.
   (*b*) Serum-simultaneous.
   (*c*) Immunisation by drug treatment of infected birds.
(2) Duration of immunity.

(1) ***Methods of immunisation.*** The following methods have been used:—

(*a*) *Refrigerated blood as vaccine.*—In order to find out how far attenuated blood is safe and effective as an immunising agent against the disease, 186 birds, in eight batches, were tested at different periods with blood which had been refrigerated for varying lengths of time, with and without resort to chemotherapy. The results are shown in Table I.

TABLE I

Immunisation with refrigerated virulent blood.

Batch	*No. of birds used*	*Period of refrigeration (days)*	*Nature of reaction*	*Mortality*	*Remarks*
1	40	9	All reacted	7	All birds treated with atoxyl
2	31	21	„ „	7	No atoxyl
3	30	25	„ „	5	No atoxyl. Deaths in heavy adult birds
4	17	29 to 30	„ „	5	Out of 22 infected, 17 left untreated with sulfarsenol, and the treated ones recovered
5	40	41	„ „	9	No drug treatment. Deaths in heavy adult birds
6	22	45	„ „	Nil	No drug treatment
7	2	50	Both showed temperature, but only one positive	Nil	„ „ „
8	4	66	All reacted	Nil	„ „ „
Total	186			27	

The results from the use of refrigerated blood, except in the case of young birds, are not encouraging. The mortality, however, was mainly among the heavy birds, especially among the Rhode Island Reds. With blood refrigerated for forty-five to sixty-six days, all birds recovered. It may be inferred, therefore, that the longer the refrigeration, the safer the blood becomes. When atoxyl or soamin is used in immunisation, even stored blood cannot be used, since these two drugs are toxic and weaken the system so that even attenuated virus may prove dangerous.

Stored blood containing dead organisms.—Blood treated by reagents to kill spirochætes was used as a vaccine by Aragao (1911) and by Marchoux and Salimbeni (1903). The writers also used refrigerated blood samples containing dead spirochætes (Table II). It was shown that dead spirochætes are harmless

TABLE II

Refrigerated blood containing dead spirochætes as a vaccine.

	Birds tested (fowl)	*Period of refrigeration (days)*	*Date of inoculation*	*Nature of reaction*	*Date and nature of retest*	*Nature of reaction*
1.	89 178	21	7/7/41	Temperature only	12/7/41 Virulent blood	Nil
2.	458 455 453 804 810	22-25	22/6/42	Nil	27/6/42 Exposed to natural infection	Three (458, 455 & 453) developed infection
3.	477 480 484 448	23	19/8/41	Nil	28/8/41 Virulent blood	No. 477 alone developed infection
4.	524 475	60	1/11/41	Nil	16/11/41 Virulent blood	Both developed infection
5.	808	95	22/6/42	Nil	27/6/42 Exposed to natural infection	Developed infection
6.	441 472 457	100	20/10/41	Nil	28/10/41 Virulent blood	All developed severe infection

Out of the seventeen birds vaccinated none reacted to the vaccine but ten developed infection on retest.

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but it is doubtful whether these give any immunity. Blood samples in which the spirochætes had been killed by the addition of oxalate also failed to produce immunity.

(*b*) *Serum simultaneous method.*—Kliger, et al. (1938), carried out extensive researches to evolve a practical method of immunisation. They used a killed vaccine (formalised cultures), as well as immune serum plus live spirochætes. They report favourably upon the serum vaccine, the resulting immunity lasting for six months. They used as a routine 0.5 c.c. immune serum followed shortly afterwards by an injection of 0.25 c.c. virulent blood. Injection of immune serum alone is said to give protection for about three weeks. One important point, i.e., the optimum time to bleed a bird for immune serum, is not mentioned.

In the present enquiries, four brews of serum were prepared and tested in doses of 0.5, 1 and 2 c.c., in sixty-six birds (both chickens and adults). Control birds were left untreated. Serum was used (*a*) simultaneously with virulent blood, (*b*) 2½ hours before, and (*c*) twenty-four hours before the injection of virulent blood. The serum was from fowls which had just recovered from experimental infection and from fowls which had recovered between 2½ to ten months previously and which shortly before bleeding had been proved immune by an injection of virulent blood. The results are shown in Table III. From these it will be seen that immune serum failed as a prophylactic.

TABLE III

			Details of Serum Test.				
Group	*No. of birds*	*Serum*	*Dose*	*Time Interval*	*Source of virulent blood*	*Reaction*	*Result*
1.	12	Brew No. 3	0.5 c.c.	Simultaneous	Fresh chicken strain	Severe and one died. Control reacted mildly	Others saved by sulfarsenol at the last stage
2.	12	„	„	„	Fresh adult strain	Severe. One control died	Birds saved by sulfarsenol
3.	10	Brew No. 2	1 c.c.	2½ hours before	„	First six days no reaction, re-infected—severe reaction	ditto
4.	10	Brew No. 1	5 c.c.	„	„	Severe late reaction, one died	ditto
5.	10 chickens (4 months old)	„	„	„	„	Reacted earlier	ditto
6.	14	Brew No. 4	10 birds, 2 c.c.; 2 birds, 1 c.c; 2 birds, nil	24 hours before	Fresh natural infection	Eight of the serum birds reacted and one died (in 2 c.c. lot). Two controls died.	Birds not treated; eleven of them recovered

In the first five batches serum neither prevented nor modified the disease; indeed, in certain cases, it aggravated the symptoms. This result was also observed by Levaditi (1904). In batch I the controls reacted mildly but the birds which received the serum reacted severely and had to be saved by administration of sulfarsenol.

(*c*) *Immunisation by drug treatment of infected birds.*—Four arsenical preparations, namely, salvarsan, atoxyl, soamin and sulfarsenol were used and of these the last three were studied in detail. The drugs were injected intra-

muscularly on a live-weight basis or in a flat predetermined dose. In every immunised batch all the birds, or at least ten where the groups were large, were closely observed as to temperature and presence of spirochætes in the blood. It was found that the second day after infection is the safest and best time for chemotherapy.

(1) *Salvarsan.*—The drug was used by Balfour (1911) in fowl spirochætosis and, though effective, was found to be toxic. Dschunkowsky (1911) injected geese with 0.075 gm. salvarsan simultaneously with virulent blood and thus prevented infection. He also found the drug to be curative. As only a small amount of the drug was available to the writers, it was only possible to experiment with thirty birds. Doses of 0.015 and 0.02 gm. were injected with promising results, all the birds recovering.

(2) *Atoxyl.*—For toxicity tests with this drug, eighteen healthy birds were divided into three equal lots. The first lot was given 0.03; the second, 0.04; and the third, 0.05 gm. per kg. live-weight for each bird. The birds did not show any rise of temperature but all evinced extreme inanition, went off their feed and remained gathered together in one corner in a stupefied condition. This was followed by greenish diarrhœa, exhaustion, a subnormal temperature and in some cases by death. The severity of the symptoms was not proportional to the size of the dose. Eight out of the eighteen died.

Altogether, sixteen batches containing 537 birds were immunised, using atoxyl in doses of 0.03, 0.04 and 0.05 gm. per kg. body-weight, the drug being given thirty-six to forty-eight hours after an infective dose of blood. Out of the sixteen batches of birds treated, only one batch (twenty birds) of about six months recovered without incident. The average mortality was 10 per cent. Mortality was highest in the group which received 0.05 gm. atoxyl and in this dose, in spite of being more toxic, was used in order to avoid having to give a second dose. Of the birds that died, more than 60 per cent. were Rhode Island Reds and most of these also showed extensive visceral gout at autopsy. Mortality also occurred in Leghorns, Minorcas and cross-breds, even among those receiving the 0.03 gm. dose.

Apart from the toxicity of atoxyl it also proved somewhat unreliable. In many cases the dosage, irrespective of the amount given, had to be repeated several times owing to persistence of the organisms.

Six healthy birds were used in a prophylactic test. All were given atoxyl at 0.05 gm. dose, three of them twenty-four hours before and three two hours before infection with the routine attenuated strain. Five birds developed the disease. Those of the first lot (twenty-four hours before) reacted severely and one died. In the second lot the reaction was much the same, two of the three showing symptoms. The experiment indicates that atoxyl in the dosage used depresses the body defences and exalts even an attenuated strain of spirochætes.

(3) *Soamin.*—In toxicity tests with this drug, ten healthy fowls were divided into two lots, the birds in each lot being given $\frac{1}{3}$ gr. and $\frac{1}{2}$ gr. respectively. All ten became dull and declined food, while one of those which had received $\frac{1}{2}$ gr. died. This one showed gouty lesions at autopsy.

In all, 106 birds in four batches were immunised by similar method as used in the case of atoxyl. Dosing often had to be repeated before the spirochætes

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disappeared from the blood. The mortality was also very high, reaching about 11 per cent. Though the blood was negative twenty-four hours after treatment, some birds died, probably owing to toxic effect of the drug. In these cases the spleen was found to be enlarged.

Mortality was highest in the White Leghorns. There appears to be an individual idiosyncrasy or some special susceptibility associated with breed, age, condition, etc. Neither atoxyl nor soamin are, therefore, satisfactory for treatment or immunisation.

(4) *Sulfarsenol.*—Of the three arsenical compounds sulfarsenol is the least toxic. The drug was subjected to a toxicity test by injecting 0.03, 0.04 and 0.05 gm. into three batches of two birds each. The only effect noted was slight dullness lasting for five or ten minutes.

As yet, there is no information as to the action and safety of sulfarsenol in fowl spirochætosis. The present experiments provide ample evidence of the importance of this arsenical in the control of the disease. The clinical condition quickly improves and no toxic effect is produced. The results suggest that a dose of 0.015 gm. per bird, irrespective of breed, weight or age, acts satisfactorily. A dose of 0.01 gm. sometimes proved insufficient. In all, 1,072 birds were immunised with sulfarsenol (Table IV). The procedure adopted was similar to that with the previous three drugs.

TABLE IV

Immunisation using sulfarsenol

Batches	*Number of birds in each batch*	*Infective material*	*Dose rate*	*Mortality*	*Remarks*
1	71	Fresh blood, routine strain	0.01 gm.	0	
2	23	Blood, 18 days refrigerated	ditto	0	
3	108	Fresh virulent blood	ditto	5	Mortality in 5 birds may be due to insufficient dosage
4	21 (chickens)	ditto	ditto	0	
5	185	Fresh blood, routine strain	0.015 gm.	0	
6	117	Virulent blood, natural case	ditto	0	
7	40	Fresh blood, routine strain	ditto	0	
8	184 (fowls) 10 (chickens)	ditto	ditto	1	Condition of chicken which died was poor
9	91 (fowls) 7 (chickens)	ditto	ditto	0	
10	20	Virulent blood, natural case	ditto	0	
11	40	Virulent blood, routine strain	ditto	0	
12	55	ditto	0.02 gm.	0	
13	100	Virulent blood, natural case	ditto	4	The four dead birds were fat
Total	1,072			10	

Note.—223 received at the rate of 0.01 gm. each, 694 at 0.015 gm., and 155 at the rate of 0.02 gm. each. Percentage of mortality is less than 1 per cent. on the whole.

To make a fair comparison between atoxyl and sulfarsenol, the following experiment was undertaken. Thirty birds were divided into two well-balanced lots, one lot receiving atoxyl at 0.04 gm. per kg. and the other sulfarsenol at a flat dose of 0.01 gm. Both drugs were given forty-eight hours after infection, when all thirty birds were showing parasites in the circulating blood. In the atoxyl group four died and all showed visceral gout at autopsy, while in the sulfarsenol group all recovered. It is certain that, in the latter group also, some birds were affected with gout,, but even in these sulfarsenol failed to cause death.

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The only instance of failure met with in using sulfarsenol was in a batch of twenty-five young chickens in a severe natural outbreak of spirochætosis, in which twenty-one of the twenty-five died. The failure may perhaps be attributed to the drug having been given too late and to the very poor condition of the chicks. On the other hand, thirty-eight chickens were experimentally infected and then treated with sulfarsenol with only one death.

The superiority of sulfarsenol over atoxyl and soamin was again clearly demonstrated in a comparative experiment on ten birds each. All the birds in the sulfarsenol group improved rapidly after treatment and even gained in weight. In each of the two latter groups two birds died.

The three arsenical compounds are easily soluble in water. Of the three, sulfarsenol seems to be more stable in solution and is undoubtedly the most active chemotherapeutic agent so far tested on fowl spirochætosis. Experience indicates that unlike as with atoxyl and soamin different batches of birds do not vary in their tolerance of this drug.

Every effort should be made in natural outbreaks to give treatment, however advanced the disease may be. Indeed, sulfarsenol may perhaps be advised in all febrile conditions in valuable laying hens, even when spirochætes have not been demonstrated. Old and fat birds are, however, unsuitable for immunisation even with this drug. An experiment, summarised in Table V, shows that sulfarsenol is not only curative but also prophylactic.

TABLE V

Prophylactic tests with sulfarsenol

Number of birds used	*Dose*	*Time between drug and infection*	*Reaction*	*Interval before test*	*Results*
3	0.03 gm.	Simultaneous	Nil	9 days	Immune
3	ditto	2 hours	Nil	ditto	ditto
3	ditto	24 hours	Nil	ditto	ditto
2	ditto	48 hours	Nil	5 days	ditto
2	ditto	72 hours	Nil	Not tested	

Control birds inoculated with spirochætes at time of immunisation and test reacted severely.

As will be seen from Table V, 0.03 gm. sulfarsenol protects birds against an infective dose of spirochætes given up to seventy-two hours previously. It is not clear, however, whether the non-reaction to the second test was due to the presence of the drug in the system or to active immunity produced as a result of the virus injection. If the latter, it may be possible to produce an immunity without the least tissue reaction, as achieved in the case of the blocked-out reaction in the serum-simultaneous immunisation against rinderpest. These points will be taken up later for investigation.

Finally, it must not be forgotten that in India no treatment which is too costly could possibly be undertaken. The cost of the three drugs, as obtained from a commercial house in Bombay, worked out per dose as follows: atoxyl, six pies; soamin, three pies; and sulfarsenol nine pies to 1½ annas per bird. This takes no account of the expense of the inoculation service. The sulfarsenol used in this work had been prepared for human use. Possibly a cheaper preparation for veterinary use could be provided if a sufficient demand for this most useful arsenical could be created.

(*To be concluded*)

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Clinical Cases

AN UNUSUAL TYPE OF DEVELOPMENTAL ERROR IN THE BOVINE

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Winchester

Subject.—Guernsey cow; multiparous.

History.—Had been in labour twenty-four hours before assistance was obtained.

Examination.—Vaginal examination revealed that the fœtal membranes were intact but that the cervix was widely dilated. The membranes were ruptured and the fœtus found to be in a posterior presentation in the dorsal position with a slightly impacted bilateral hock flexion.

A dead bull calf was delivered and was found to have a patent umbilical ring of about four inches in diameter. Through this ring, omentum, part of the duodenum, and a large part of the abomasum projected. These organs were fully exposed and there was a complete absence of skin-covering in this area.

Comment.—Williams, in "Diseases of the Genital Organs of Animals," 2nd Edition, 1939, refers to this type of abnormality as a schistocormus and states that, according to his observations, such defects are largely accompanied by malformation in the urinogenital system. Unfortunately in this case no examination was made of this.

DISPLACEMENT OF OS CALCIS AND ASTRAGALUS IN A BITCH

By EDGAR von LUSTIG-LENDVA, M.A., M.R.C.V.S.,
Hendon

THE subject was a fox-terrier bitch aged eight years. She was lame after a sudden slip. There was collapse of the right hind limb, allowing the hock to approach the ground. On examination there was no rupture of the tendo Achilles but pressure on the lower extremity of the hock caused the os calcis to rotate backwards and outwards. The tarsus was put in cellona plaster to obtain fixation of the joint.

The plaster was removed after three weeks and no improvement was seen. An X-ray was taken to ascertain the extent of the damage. This X-ray showed displacement of the os calcis and astragalus. The two bones were still firmly bound together and their relation to the tibia was almost normal. They were displaced from their normal position on the scaphoid and cuboid. No doubt the cause was a rupture of the interosseous ligaments. These ligaments are very short and strong, and such an injury must be very rare. Dislocation of the scaphoid is a common injury, especially in the racing greyhound. In this case the scaphoid is in its normal position.

Operation for ankylosis was refused by the owner, so a leather and aluminium cuff was made to fit the joint, with which the dog now walks quite easily.

PARASITIC ANEURYSMS IN A YOUNG FOAL

By MYRA L. BINGHAM, M.R.C.V.S.,
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Subject.—Filly foal, born in April, 1942.

History.—Although the foal had had intermittent attacks of scouring, no notice was taken of them until July 12, 1942, when a new stud groom, on taking its temperature during one of these attacks, found it to be 105 deg. F.; Mr. J. F. D. Tutt was then called in. The diarrhœa ceased after forty-eight hours, but the temperature continued to fluctuate from hour to hour throughout the illness, sometimes reaching 105.5 deg. F. or more. There were no other symptoms and at no time did the foal go off its feed, which consisted of meadow hay in addition to the dam's milk. A rectal swab submitted for examination to the Research Institute in Animal Pathology, The Royal Veterinary College, on July 16, revealed the presence of *Bact. coli,* but no salmonella organisms were found. The possibility that it might be a case of pyæmic nephritis was considered but subsequent examinations of samples of urine did not reveal any significant findings. The mare's milk was found to be bacteriologically normal and there was no response to M and B 693 intramuscularly or to sulphonamide orally. Dimol was then given for a period and five doses of a mixed bacterin containing *Bact. coli.* On July 27 the foal showed signs of stiffness across the loins and at about the same time a constant trickle of urine was observed when it was lying down. The temperature still varied from 104 deg. F. at night to 103 deg. to 102.8 deg. F. in the morning, and vice versa. In blood films examined on August 12, no eosinophils were found; otherwise the leucocyte count was normal. On August 14, Mr. Walker, of Slough, in consultation with Mr. J. F. D. Tutt, suggested a strongyle infection, and on August 20 a sample of fæces was examined for worm eggs at the Institute. The average of four counts made by a modified Stoll dilution counting technique was 6,750 *Ascaris equorum* eggs per gramme of fæces. No strongyle eggs were found either by this method or by a concentration centrifugal flotation method. The foal's condition remained unchanged until 5 p.m. on August 20, when it suddenly went down and had to be assisted to its feet. Following a subcutaneous injection of strychnine ¼ grain the animal was able to get up unaided and suck the mare. Death occurred early on August 21.

Post-mortem findings.—There was intense inflammation of the bowels, a portion of which was adherent to the abdominal wall. Both kidneys and a mass of tissue involving the kidneys and the adherent portion of the bowel were forwarded to the Institute.

Pathological report.—The left kidney showed an irregular area of infarction of the cortex, with obvious necrosis extending into the cortex for about 1 cm. There were also numerous other dark purplish areas which were probably lesions of early infarction. The right kidney was congested but no infarction was seen macroscopically. These areas of infarction appeared to be due to thrombosis of the renal artery secondary to aneurysm and thrombosis of the aorta and anterior mesenteric artery. Both renal arteries contained a thrombus.

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It is possible that aneurysms of the renal arteries existed between their origins and the kidneys, but only the intrarenal portions of the artery were available for examination.

The "mass of tissue" measured about 23 x 14 x 12 cm. and comprised the posterior vena cava, parts of the aorta and anterior mesenteric artery and masses of dense fibrous tissue. Dissection of the arteries revealed the following: The presence of thrombus in the aorta and one of the left branches of the anterior mesenteric artery which, in addition, contained a few *Strongylus vulgaris* larvæ. Large aneurysms involved the root, right and middle branches of the anterior mesenteric artery, in which was much thrombus and many *S. vulgaris* larvæ.

Discussion

The interesting features of this case are the age of the subject, the extent of the parasitic infection and the absence of strongyle eggs in the fæcal egg count. The possibility of prenatal infection in foals is suggested by Foster and Clark (1937), who found verminous aneurysms in two of five foals under one month old in Panama. They also found aneurysms in eleven out of twelve foals of one to six months of age and in all of six animals of seven to twelve months of age. Nieberle and Cohrs (1931) quote Pöppel, who recorded an aneurysm from a ten days old foal, and Kikuchi, who found one in an animal thirty-eight days of age. Nieberle and Cohrs state that new-born foals show no aneurysms. Wetzel (1942), on the other hand, does not consider that prenatal infection is possible and maintains that eggs found in the fæces of foals up to six weeks of age have most probably been ingested and passed out unchanged. By experimental infection of a four months old foal he has determined the interval between ingestion of the infective larvæ and sexual maturity to be 196 days.

Although similar cases have been recorded in young animals, the size of the aneurysms and the large amount of reactionary tissue seen in this case were very striking.

It is important to stress that the fæcal egg count merely indicated a moderately heavy infection with *Ascaris equorum*. Since it is generally believed that the length of time taken by *S. vulgaris* females to reach maturity and to begin egg-laying is about twelve weeks after oral infection, this could account for the fact that no strongyle eggs were found in the fæces when, in fact, a considerable number of *S. vulgaris* were found at post-mortem examination in the aneurysms.

I am indebted to Mr. J. F. D. Tutt, whose case this was, for supplying the details of the clinical history and post-mortem material, and for his co-operation in allowing this record to be published.

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Abstracts

URINARY CALCULI IN SHEEP*

By W. M. BEESON, Ph.D., J. W. FENCE, M.S., and G. C. HOLM, M.S., D.V.M.

A REVIEW of the literature reveals that the underlying cause of the disease has not been determined. Since the problem is of practical importance to the sheep industry and of fundamental value to the science of animal production, experiments were conducted in an attempt to determine the cause of urinary calculi.

Experimental diets high in linseed and cottonseed meal, calcium, magnesium, or calcium and phosphorus fed to wether lambs, with and without an adequate source of vitamin A, did not lead to the formation of urinary calculi within 150 to 160 days. Elemental sulphur or added silica did not lead to any observable effects whatever.

Vitamin A deficiency was eliminated from having any effect on the formation of urinary calculi under conditions observed in this study. Under certain conditions, wheat bran was found to lead to the formation of urinary calculi.

Individual variation in the absorption and excretion of dietary mineral elements was found to be worthy of important consideration in the etiology of urinary calculi formation.

The occurrence of an apparently rare siliceous type of calculi observed in this study was attributed to a hyperexcretory state in which urinary magnesium plays an important role in establishing the urinary silica solubility limitations.

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CO-ORDINATED TRIALS WITH PHENOTHIAZINE AGAINST NEMATODES IN LAMBS

Imperial Agricultural Bureaux Joint Publication No. 4, April, 1943

THIS publication is a report on co-ordinated trials in the use of phenothiazine as an anthelmintic in sheep. The trials were carried out at four centres at the instance of the Agricultural Research Council and the report has been produced as a special publication by the Imperial Bureau of Animal Health and the Imperial Bureau of Agricultural Parasitology (Helminthology).

The preface explains the reason for the trials. "In view of certain discrepancies in the published reports on the use of phenothiazine as an anthelmintic, and the fact that different criteria have been employed by different workers in assessing its efficacy, the Agricultural Research Council decided to institute a series of co-ordinated trials at several centres, so planned as to give results that would be susceptible of adequate statistical analysis, and would afford answers to certain specific questions." This publication is the report of the planned trials. The centres participating were (1) the Institute of Agricultural Parasitology, St. Albans; (2) the Department of Zoology, University of Edinburgh, and the Animal Diseases Research Association, Moredun, Edinburgh; (3) the Institute of Animal Pathology, Cambridge; and (4) the Veterinary Laboratory (Ministry of Agriculture and Fisheries), Weybridge. The Galton Laboratory, University College, London, was largely responsible for the statistical examination of the data as well as for assistance in the planning of the trials.

The report consists of six sections, including a summary and conclusions, together with seven appendices and a reference list. Section I is a historical review and in it are references to the findings of many of the numerous publications in the use of phenothiazine, especially in sheep. Section II outlines the purpose of the trials and discusses the agreed details of techniques, etc. Concerning the former, it is stated: "Broadly speaking, in the present trials information was required on two different topics: (i) the effects of the drug on particular species of worms, and (ii) the resulting net effect upon the lambs themselves. Light on the former and more academic topic would be shed by counts of eggs and worms; the problem of reinfestation, inevitable in a flock at pasture, would be solved by keeping the lambs inside, and the experiment need last only a few weeks. On the other hand, the second and more practical topic would best be served by the periodical weighing of lambs over a period of many weeks, the lambs being kept at pasture under natural conditions." The above quotation explains the general outline of the experiment. The section also deals with the composition of the drug used at all the centres, both in fluid and tablet form; the method of administration; the dosage (0 (controls), 5, 10, 20, 30, 40 and 50 gm. per lamb in their various lots at each centre); the selected lambs ("Each centre, therefore, endeavoured to buy the necessary number of lambs with a level of infestation of about 1,000 eggs per gramme of fæces"); the randomisation of the lambs in the trials; the management of the lambs (feeding); the time-table of the experiments; the weighing of the lambs; the technique of counting worm eggs used throughout the trials; the

technique of counting the worms in the stomach and intestines of the lambs (all the lambs were slaughtered at the completion of the trial). Section III deals with the findings at each of the four centres (the actual data, weights egg and worm counts are given in the appendices). In Section IV the results are statistically analysed; the technical details of the methods are given in the appendices. A summary and conclusions follow. The gist of the findings can be gleaned from the conclusions, which are as follows:—

(1) Phenothiazine has been tested in single doses against sheep nematodes in co-ordinated trials involving some 280 lambs at a number of centres in Great Britain, using lamb weights, egg counts, and worm counts as criteria of efficacy. Full details are given of the methods used, and it is shown that egg and worm counting techniques are satisfactory.

(2) The unit flock, omitting reserves, consisted of twenty-eight lambs given the following doses of phenothiazine in batches of four: 0 (controls), 5 gm., 10 gm., 20 gm., 30 gm., 40 gm., and 50 gm. In each batch, except the controls, two lambs received the drug as compressed tablets and two as powder made into a drench with water. About 2 per cent. sodium cetyl sulphate was used as a wetting and dispersing agent in both cases.

(3) Four of these unit flocks, maintained inside for six weeks to avoid reinfestation, were tested at Cambridge, Weybridge, St. Albans and Edinburgh; they supplied information mainly on egg and worm counts.

(4) Six unit flocks kept outside for sixteen weeks under natural conditions and tested at St. Albans, Edinburgh, and four commercial farms near Weybridge, supplied information mainly on the net effects of treatment on growth of lambs.

(5) The evidence of the inside flocks indicated that the greatest relative reduction in egg count was achieved by the lowest dose used (5 gm.), higher doses reducing the count further to a relatively small extent and irregularly. From the worm counts it appeared that the marked susceptibility of *Hæmonchus* to a 5-gm. dose was largely responsible for this effect. With most intestinal worms the response was less marked but continued up to the highest dose used. Anomalous responses were obtained from *Strongyloides, Trichuris, Nematodirus* and *Moniezia,* the last two actually showing *increased* counts with higher doses.

In an experiment lasting six weeks, with the lambs kept under conditions precluding reinfestation, an actual increase in any species is practically impossible. If these apparent increases are judged significant, the reason must be that the normal elimination of these worms is reduced.

(6) The evidence of the outside flocks, maintained for sixteen weeks, was that, while egg-count reductions had been obliterated by reinfestation (except in flocks grazed on unusually clean pastures), there was a relatively large weight response which increased roughly in proportion to dosage up to the highest dose used. This response averaged forty-eight times the weight of phenothiazine administered. The general impression here (viz., that the higher the dose (within the range explored), the better the result), indicated that, judging from the "inside" worm counts, this result must be ascribed to the reduction not merely of stomach worms but also of certain intestinal worms usually considered of little clinical importance.

(7) A detailed analysis of the data relating to the outside flocks showed

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that, within the six flocks tested, the weight responses were not affected by the initial degree of infestation (as measured by egg counts) with which each lamb entered the experiment. There is thus no confirmation of the suggestion that heavily infested lambs respond less well than lightly infested lambs. If it can be assumed that these lambs were not greatly above the average level of infestation for lambs of the same age in this country, then it is likely that the average flock will respond at least as well as these.

(8) There is no evidence of any difference in efficacy between tablets and drenches containing the same weight of phenothiazine beyond a suggestion from the worm counts (which are too variable for exact statistical treatment) that drenches may be slightly more efficacious against stomach worms only. If so, the explanation may be that the particles of phenothiazine in a tablet are not fully dispersed until they are mixed with the host's bile salts.

(9) This report contains two new developments of existing statistical procedures: (i) the method for transforming the sigmoid "dosage mortality" curve to a straight line is extended to cover data like egg counts, where expectations are calculable from prior observations; (ii) the method of analysing multiple regressions by the use of the "covariance matrix" is developed so as to cope with a series of samples, which are pooled with respect to some variates, but treated independently with respect to others.

[Copies of the report may be purchased from Central Sales Branch (Imperial Agricultural Bureaux), Agricultural Research Building, Penglais, Aberystwyth, Wales. Price: 3s. 6d. per copy.]

SWINE INFLUENZA

A new concept in the epidemiology of virus infections

SWINE INFLUENZA was first recognised in the United States after the great epidemic of human influenza in 1918. In 1936 Shope[1] summarised his work showing that it was a disease of complex ætiology, the syndrome recognised as swine influenza being due to the combined action of the influenza virus and *H. influenzæ suis*. Blakemore and Gledhill[2] have described an influenzal disease of pigs in this country resembling the condition in the U.S.A. but with a somewhat more varied bacterial flora.

Endeavouring to discover what happened to the virus during the summer months when swine influenza is unknown, Shope demonstrated, in preliminary experiments, that the embryonated lungworm ova in the fæces or bronchial exudate of infected pigs are capable of carrying the swine influenza virus. These ova are ingested by earthworms in which the lungworms in question pass some stages of their life cycle. If, at a later date, a susceptible pig eats these earthworms, the larvæ, still carrying virus, reach the lungs and the mature lungworms develop. Even this, however, is not enough to produce swine influenza. The virus lies harmlessly in the worms until some provoking stimulus lights up the infection, in an unknown way, and the pig develops swine influenza.

Shope has now reported work on a much larger scale carried out during the last three years.[3] Metal drums of fifty-gallon capacity were sunk in the ground and half-filled with earth; lungworm ova and minced adult worms

were added and covered with two to three inches of earth; 200 to 400 earthworms were then placed in the drum. This process was repeated at intervals until the drum was nearly full. In all, ninety-eight transmission experiments were carried out, ten to fifty or more of the infected earthworms from the drum being fed to each of 216 swine. The most effective "provocative stimulus" consisted of multiple intramuscular injection of *H. influenzæ suis*, but positive results were only obtained in eight-six swine, most of these animals developing clinically characteristic influenza. Many other stimuli were tried and were ineffective, but in seven experiments animals developed the virus infection without the application of any known stimulus; the time of this apparently "spontaneous" provocation may have corresponded to that at which the natural immune response of the swine to the lungworms developed, and it is suggested that the stimulus was an allergic reaction to the lungworms.

The whole series of experiments showed that it was easiest to produce the disease in January, February and April; all attempts in May, June, July and August were entirely negative, although pigs were infected with lungworms known from tests at other times of the year to be carriers of masked influenza virus. Masked virus was demonstrated in lungworm ova from convalescent swine, in which the normally infective virus could not be demonstrated, and it has been shown to persist for at least thirty-two months in lungworm larvæ.

In a field study[4] Shope demonstrated swine influenza virus in lungworm larvæ from earthworms dug on mid-western farms; he has also shown that the disease can be provoked in pigs taken from farms in this region during interepidemic periods, and subsequently kept in rigid laboratory isolation, by the intramuscular injection of *H. influenza suis*.

Shope believes that the lungworm is the agent responsible for the perpetuation of swine influenza virus from one epidemic to the next and suggests that the popular conception of the epidemiology of swine influenza may be quite incorrect; it may be that in a natural epidemic most cases do not arise from direct exposure to diseased animals but represent instances of provoked masked virus infections, and that the spread of influenza from animal to animal, though readily accomplished, is the exception rather than the rule. This conception would explain the known epidemiological facts, the onset of an epidemic not being determined by the acquisition of the causative virus, in a masked form, but by meteorological or physical influences which favour activation of the virus.

There is much information concerning the importance of the carrier state in relation to bacterial diseases, but we know far less about the role of carriers in the epidemiology of virus infections, although they are assumed to exist, and many latent virus infections have been demonstrated in experimental animals. Shope's work introduces a new concept into the epidemiology of virus infections and revives—admittedly, in a modified form—the old idea of the spontaneous origin of disease. The epidemiology of some other virus diseases is still poorly understood and this work may provide a clue worthy of further investigation. The origin of outbreaks of *Brustseuche* and influenza in horses[5] is often obscure and we do know that stallions may carry the virus of influenza for long periods and transmit it by copulation.

XUM

In order to explain the ætiology of cancer some workers have postulated the existence of a virus, common to all tumours, and an inanimate agent or "specific factor" which provides the immediate stimulus to tumour formation. Andrews (1939) drew attention to the possible relationship between latent virus infections and cancer, but Hadfield and Garrod (1942) concluded that the facts do not warrant even a provisional acceptance of the virus theory in considering the ætiology of cancer. Shope's work on swine influenza has, of course, no direct relationship to cancer, but it is an excellent example of a virus which can remain dormant in the animal tissues for long periods and then produce overt disease when the appropriate stimulus is applied under suitable conditions.

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Obituary

Sir James Ralph Jackson, M.R.C.V.S. On August 2, 1943, Sir James Ralph Jackson died at Torquay at the age of 71 years. Most of his professional life was spent in the service of the Ministry of Agriculture and Fisheries, which he joined soon after graduating from the Royal (Dick) Veterinary College in 1897. He became Chief Veterinary Officer in 1926, and retired on reaching the age limit in 1932. A knighthood was conferred on him in 1929.

Correction

In our issue of July, 1943, the word BIPAP in Messrs. Evans Sons Lescher & Webb's advertisement should read *BIBAP*.

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